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ANNUAL PROGRESS REPORT

August 1, 1962 - May 28, 1963

C. M. Pomerat

Pasadena Foundation for Medical Research
Pasadena, California

CYTOTOLOGICAL ASPECTS OF INJURY AND REPAIR

Grant No. DA-MD-49-193-63-G80

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ABSTRACT

1. Preparing Institution: Pasadena Foundation for Medical Research
2. Title of Report: Cytological Aspects of Injury and Repair
3. Principal Investigator: C. M. Pomerat
4. Number of pages and date: 8 pages - May 28, 1963
5. Grant Number: DA-MD-49-193-63-G80
6. Supported by: U. S. Army Medical Research and Development Command
Department of the Army
Washington 25, D. C.

Studies on the effect of anesthetics on clusters of human tracheal epithelial cells have been extended to lesions in the intracellular environment. Considerable progress has been made in the cultivation of cells from dental pulp and membrane behavior of the KB cell line. Additional facts have been accumulated regarding the mechanism of Actinomycin on mitosis. The anti-inflammatory properties of a water insoluble liver extract as well as organic and inorganic selenium have been studied with experimentally produced granuloma pouches.

New attacks have been made in the study of injury and repair following the treatment of cells with staphylococcus and tetanus toxins. Efforts to demonstrate a possible relation between Bence Jones protein and neural mechanisms thus far have not been successful.

The cultivation of endothelial cells and of epithelial systems from the lining of the female reproductive system following their disaggregation with trypsin has led to two publications which point the way for further explorations in the physiology of cardiovascular structures and of all hollow organs.

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I. Mechanism of Anesthesia

We were very fortunate in having interested Dr. William C. Yang, Associate Professor of Pharmacology of the University of Southern California School of Medicine, in this project. Collaborating with our Mr. Fred Strasser, Dr. Yang has recorded cytological evidences of injury following the use of anesthetics and various other drugs pertinent to this project. A report of this effort is in preparation.

II Morphological Studies

The important findings in this area have been in the field of dental cytology and pathology. A study of the KB cell line, which originated as a squamous cell tumor of the oral cavity, is reported in reference #2. This dealt with the membrane activity (zeiosis) of cells in relation to the nucleus and endoplasmic reticulum.

We have aided a second-year dental student, Mr. Larry Smith, who had a fellowship last summer provided by the San Gabriel Valley Dental Society. Mr. Smith was successful in the cultivation of odontoblasts and has prepared an excellent series of cinematographic records of the activities of these cells. While this work is not yet ready for publication, it was presented at a major dental meeting on May 21, 1963.

Using the know-how which was acquired in connection with our studies on the effect of procaine with Dr. Yang with the use of perfusion chamber technique, we have described the cytopathology resulting from the application of procaine hydrochloride to human gingival epithelial cells as well as to connective tissue elements from dental pulp. The value of such work lies in the opportunity to assess new local anesthetics developed for dental surgery.

III. Differential Action of Chemicals on Cell Organoids

Dr. Donald Rounds has continued to accumulate data on the effect of Actinomycin on various cell lines in relation to the potentiation of anti-tumor activity of this drug.

An observation that cells treated with the anti-cancer drug, Actinomycin, survived for a longer period of time in complete medium than when treated in a balanced salt solution led to a study

of the combination of Actinomycin and adenine-containing compounds. It was hoped that the results of such a study would lead to a better understanding of the mechanism of action of Actinomycin and, perhaps, enhance the differential toxicity of the drug.

It was found that adenine-containing compounds promoted survival of cell lines during a 48-hour treatment with 1 gamma/ml Actinomycin P2. The compounds could be arranged in order of their effectiveness, as follows: adenosine > AMP > ADP = FAD = riboflavin > ATP = adenine. Adenine and ATP had little effect on survival. Adenosine produced a two-fold survival in HeLa cells, four-fold in KB cells and a five-fold increase in cells from conjunctiva and human lung lines.

The control rate of mitosis appeared to be maintained in the cell lines of nonmalignant origin (conjunctiva and lung) when Actinomycin and adenosine were administered simultaneously, but this treatment produced a significant depression of the HeLa and KB mitotic indices.

The effect of Actinomycin on the reduction of nucleolar mass was not affected by the addition of adenosine. It was thought that adenosine survival was not mediated through an enhancement of RNA synthesis. The prevention of mitochondrial fragmentation with adenosine suggests that oxidation-phosphorylative pathways may be maintained. The lack of response to ATP treatment may be related to differences in the rate of membrane permeability of phosphorylated compounds. This possibility is under investigation.

IV. Multiple Myeloma

Clinical colleagues have observed that multiple myeloma patients with Bence Jones protein patterns showing an elevation in the gamma globulin region sustained relief from pain when treated with cycloleucine (NSC 1026). Elevations of other globulin peaks showed no such association with pain relief. An attempt was made to establish a test system which would yield quantitative data pertinent to this observation.

Serum from patients having Bence Jones protein was collected together with suitable Bence Jones-free sera from control patients. Eagle's medium was reinforced with 10 per cent serum of both types for use in the cultivation of dorsal root ganglia with the hope of determining whether or not any aspect of the regeneration of sensory nerves might be influenced by the presence of the Bence Jones protein. While only a few experiments have been performed, the results thus far have been negative.

V Wound Healing

As stated in our research grant application dated May 29, 1962 Mr. Joel Adams is accumulating data for his Ph. D thesis in this laboratory. During the past few months Mr. Adams has perfected his technique for cloning cell lines according to the original suggestion of T. T. Puck. He has accumulated relatively large quantities of HeLa cells and has worked out the phenolic extraction technique for the isolation of DNA. He has also demonstrated that such purified DNA enters HeLa cells with the use of the acridine orange fluorescent technique. His goal is to alter the genome of a cell line with respect to some easily definable metabolic marker.

Considerable attention has been focused on changes in the character of cell populations as measured by marker chromosomes. For several years we have been following the behavior of a synovial cell line derived from a patient with "degenerative arthritis." A report on the chromosomes of this line is given in the bibliographic citation 1

Dr. M. Roberts has received aid, in part, from this grant in pursuing his studies on anti-inflammatory agents. It has been possible to demonstrate that the factor which had therapeutic possibilities was contained in a water insoluble fraction of liver. Beyond this, it was established that not only organic but inorganic selenium might be responsible for the anti-inflammatory effect which could be measured with experimentally produced granuloma pouches. These results appear in references 3 and 4.

From the cytological point of view, the effect of selenium is being examined with a variety of techniques. It has been found, with the use of the Coulter cell counter, that between 5 and 10 gamma per milliliter of sodium selenite all cells die within 72 hours. Employing a larger dose (100 gamma) but for a period of only five hours, cinematographic studies have revealed progressive changes in the mitochondria extending from a beaded appearance to the formation of numerous large vesicles. Using a Beckman Spinco physiological gas analyzer, we are attempting to correlate selenium injury to mitochondria with a temporary increase in oxygen consumption.

A very important new chapter for experimental studies has been opened with the use of the technique of trypsinizing hollow organs so as to disaggregate their lining cells. The paper by Pomerat and Slick deals with the behavior of endothelial cells collected from the rabbit aorta, while Dr. Carlo Valenti, who worked in this laboratory for some three months, received support, in part, from this grant in the preparation of a study dealing with the epithelial cells from

the female reproductive tract harvested as a result of introducing trypsin in the cavity of various organs from this system (see refs 5 and 7). It has been suggested in these papers that there is an important opportunity for the analysis of tissue repair with the use of this method. The cavity of all hollow organs, particularly those obtained as surgical specimens, can provide epithelial cells whose behavior can be followed with *in vitro* techniques. Suggestions have been made for future work directed at the elucidation of reparative, endocrine control and neoplastic mechanisms.

Dr. José Simões, who is Consultant in the Curry Cabral Hospital, Medico das H. C. L., in Lisbon, Portugal, studied with us for a period of seven months as a fellow of the Fundação Calouste Gulbenkian. After learning basic tissue culture methods, he directed his attention at the mechanism of action of tetanus toxin on living cells. He encountered two major difficulties in obtaining: (1) preservative-free toxin of sufficient strength, and (2) toxin which did not contain spores. The removal of spores from test material with various filters proved difficult, but the obstacle was overcome with high speed centrifugation. On the positive side, he was rewarded with the preparation of extraordinary time-lapse cine records showing the ingestion and subsequent fate of tetanus spores by cells. Unfortunately, this work has not yet been brought to a publishable stage, but it is hoped that this goal will be met in the near future.

A publication which had been reported in our previous renewal as "in press" has been published (item 6). This study, calling attention to cytological factors involved in injury and repair following staphylococcus toxin and antiserum, provoked very considerable interest. We have accumulated more than 300 reprint requests for this publication.

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